Supplementary Table 1

Protein	Locus	Coverage (%)	Known Chk1 interactor
Chk1	NM_001274	71.2	-
Claspin	NM_022111	7.7	Yes [a]
14-3-3	NM_139323	48.4	Yes [b]
Protein Phosphatase 2C	NM_002706	38.2	Yes [c]
PCNA	NM_022381	13.0	No

Table S1. Summary of mass spectrometry data showing proteins identified from FLAG-Chk1 IPs that were not present in control FLAG IPs. The percentage coverage of the proteins is also shown.

[a](1,2)
[b](3,4)
[c](5)

Supplementary References:

1. Chini, C. C., and Chen, J. (2003) *J Biol Chem* **278**, 30057-30062
2. Kumagai, A., and Dunphy, W. G. (2003) *Nat Cell Biol* **5**, 161-165
3. Chen, M. S., Ryan, C. E., and Piwnica-Worms, H. (2003) *Mol Cell Biol* **23**, 7488-7497
4. Jiang, K., Pereira, E., Maxfield, M., Russell, B., Goudelock, D. M., and Sanchez, Y. (2003) *J Biol Chem* **278**, 25207-25217
5. Lu, X., Nannenga, B., and Donehower, L. A. (2005) *Genes Dev* **19**, 1162-1174

Supplementary Figure 1

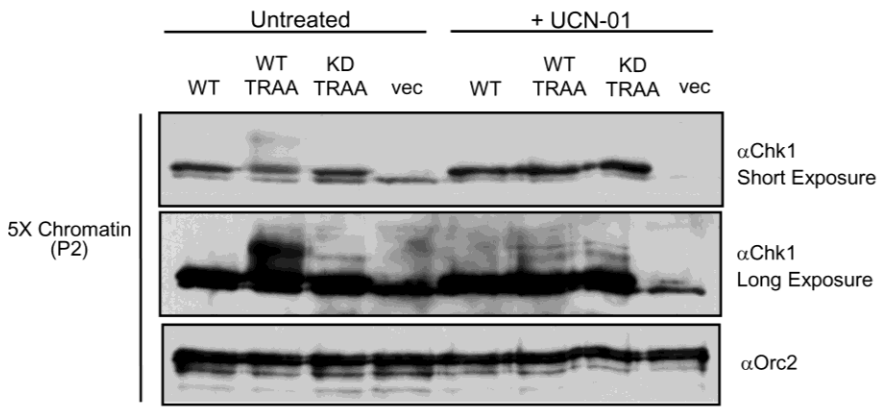

Figure S1. The altered mobility of FLAG-Chk1^{TRAA} on chromatin is a product of auto-phosphorylation. FLAG-Chk1^{TRAA} displays a hyper-shifted form on chromatin (P2) which is significantly reduced following treatment with UCN01 and is not present in kinase-dead FLAG-Chk1$^{KD-TRAA}$. HeLa cells were transfected with FLAG-Chk1 as indicated above the panel and left untreated or treated with 300nM UCN-01 for 3 hours. Orc2 was used to verify loading of the chromatin fraction.

Supplementary Figure 2

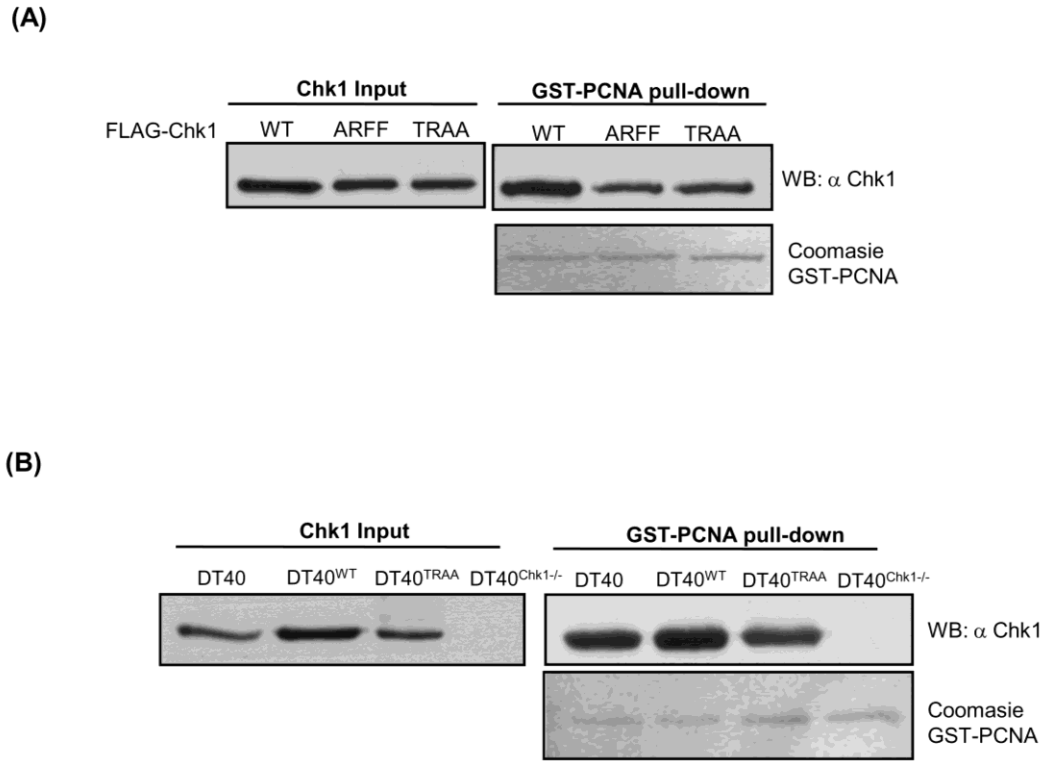

Figure S2. Mutations in the PIP box of full-length Chk1 do no abrogate PCNA binding.
(A) GST-PCNA was assessed for the ability to interact with full length FLAG-Chk1^{WT}, FLAG-Chk1^{ARFF} and FLAG-Chk1^{TRAA} expressed in HeLa cells. In the context of the full-length Chk1 protein, mutation of conserved hydrophobic residues in the PIP box does not abolish PCNA binding. (B) GST-PCNA was probed for the ability to bind Chk1 from DT40 cells and DT40$^{Chk1\ -/-}$ cells reconstituted with GgChk1^{WT} (DT40^{WT}) or GgChk1^{TRAA} (DT40^{TRAA}). DT40$^{Chk1\ -/-}$ cells were included as a control. As with human constructs, mutations within the PIP box do not abolish PCNA binding to the full length Chk1 protein.